Eur. J. Clin. Chem. Clin. Biochem.
Vol. 31, 1993, pp. 625–631



# A Trypsin Sensitive Stromelysin Isolated from Rheumatoid Synovial Fluid is an Activator for Matrix Metalloproteinases

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(Received April 16/June 21, 1993)

**Summary:** The processing of synovial fluids of patients suffering from rheumatoid arthritis led to the characterization of a neutral metalloproteinase with polymorphonuclear leukocyte progelatinase and polymorphonuclear leukocyte procollagenase activating properties. The activator exhibits a relative molecular mass of $M_r$ 27 000 and is an active form of stromelysin. Thus, it reacts specifically with antibodies raised against human stromelysin, splits polymorphonuclear leukocyte progelatinase in a manner characteristic of stromelysin, and is inhibited by EDTA as well as by a tissue inhibitor of metalloproteinases (TIMP-2).

The activator shows a high specificity for the matrix metalloproteinases, polymorphonuclear leukocyte progelatinase and polymorphonuclear leukocyte procollagenase. It shows only weak hydrolysis of casein and gelatin, and it does not activate fibroblast $M_r$ 72 000 progelatinase. Brief treatment with trypsin does not lead to a significant change in the activator's relative molecular mass, but induces a rapid loss of its activating activity for polymorphonuclear leukocyte progelatinase, while its proteolytic activity against the synthetic substrate, N-(2,4)-dinitrophenyl-Pro-Gln-Gly-Ile-Ala-Gly-Gln-*D*-Arg, is increased about 3-fold. The same tryptic treatment does not affect the activator's proteolytic activity towards casein and gelatin.

## Introduction

Matrix metalloproteinases play an important role in remodelling of the extracellular matrix (1, 2). Since the structural elements of the extracellular matrix are all substrates, the activity of these enzymes must be strictly regulated. In certain diseases, e. g. rheumatoid arthritis, these control mechanisms do not function properly, leading to destruction of the extracellular matrix. In the special case of rheumatoid arthritis, destruction of the articular cartilage finally leads to the destruction of the whole joint.

Matrix metalloproteinases are secreted as latent proenzymes by different cell types, such as fibroblasts, macrophages and polymorphonuclear leukocytes, in response to certain stimuli. An important regulatory mechanism involves activation of the latent enzymes in the extracellular space. *In vitro* metalloproteinases can be activated by organomercury compounds. Most matrix metalloproteinases are also activated by trypsin.

It is assumed that the physiological activator for the matrix metalloproteinases, collagenase[1]) and stromelysin[1]), might be plasmin[1]). Activated stromelysin (formerly called proteoglycanase) has a rather broad spectrum of activities; it hydrolyses not only proteoglycan, but also other components of the extracellular matrix, such as laminin, fibronectin, gelatins and collagen types III, IV, V and IX, and it is

[1]) Stromelysin EC 3.4.24.17
Polymorphonuclear leukocyte gelatinase EC 3.4.24.35
Fibroblast collagenase EC 3.4.24.7
Polymorphonuclear leukocyte collagenase EC 3.4.24.34
Trypsin EC 3.4.21.4
Plasmin EC 3.4.21.7

also reported to be a "superactivator" for plasmin-activated collagenase, enhancing the collagenase activity about 10-fold (3—5). Recently, it was reported that stromelysin is an activator for polymorphonuclear leukocyte progelatinase (6). Thus, stromelysin is not only an important proteinase with regard to its activity towards components of the extracellular matrix, but seems to play a key role in regulation of the activities of other matrix metalloproteinases.

Here we report some newly discovered properties of stromelysin (isolated from rheumatoid synovial fluid) that appear to extend the regulatory involvement of this enzyme.

## Materials and Methods

### Materials

Rheumatoid synovial fluid originated from patients of the Rheuma-Klinik, Immanuel-Krankenhaus, Berlin. Buffy coat was kindly supplied by Deutsches Rotes Kreuz, Berlin. Ultrogel AcA 44, blue-Trisacryl, heparin-Ultrogel and phorbol myristate acetate were purchased from Serva, Heidelberg. Gelatin-Sepharose was prepared in our laboratory. Sepharose 4-B was obtained from Pharmacia, Freiburg. 4-Aminophenylmercury acetate, plasmin (3 U/mg) and molecular mass markers for gel electrophoresis were obtained from Sigma. Protein concentrations were determined with the bicinchoninic acid reagent (Pierce, USA) with bovine albumin as standard.

### Enzyme assays

Matrix metalloproteinase activities were assayed with the synthetic substrate N-(2,4)-dinitrophenyl-Pro-Gln-Gly-Ile-Ala-Gly-Gln-*D*-Arg (dinitrophenyl-labelled peptide) (7). Activity against protein substrates, such as gelatin and casein was assayed by the fluorescamine method (8). Activator activity was analysed by incubating the activator with polymorphonuclear leukocyte progelatinase for 30 min at 37 °C in the presence of the dinitrophenyl-labelled peptide. The amount of substrate hydrolysed by the activated polymorphonuclear leukocyte gelatinase was taken as a measure of activator concentration.

### Isolation of matrix metalloproteinases

Polymorphonuclear leukocyte progelatinase and polymorphonuclear leukocyte procollagenase were purified from buffy coat after stimulation with phorbol-myristate acetate. The secreted metalloproteinases were purified essentially as described (9, 10). The $M_r$ 72 000 progelatinase and the $M_r$ 72 000 progelatinase-TIMP-2 complex were isolated according to l. c. (11).

### Activator purification

To 100 ml of rheumatoid synovial fluid, saturated ammonium sulphate solution was added to 10% saturation. After 60 min in the cold the precipitate was removed by centrifugation at 40 000 *g* for 30 min, and the clear supernatant was adjusted to 50% ammonium sulphate saturation. After 24 h at 4 °C the precipitate was collected by centrifugation at 150 000 *g* for 20 min, washed twice with 60 ml ammonium sulphate solution (313 g/l) and centrifuged again. The sediment was dissolved in 20 mmol/l imidazole-HCl, pH 6.1, 0.5 g/l Brij 35, 0.5 g/l $NaN_3$ and dialysed against 20 mmol/l imidazole-HCl, pH 6.1, 5 mmol/l $CaCl_2$, 0.5 g/l Brij 35, 0.5 g/l $NaN_3$ (buffer A) followed by dialysis against buffer A. The sample was chromatographed on carboxymethyl-trisacryl (5.5 cm × 11 cm) equilibrated in buffer A. After washing with buffer A, bound protein was eluted with buffer A containing 0.5 mol/l NaCl and stored at −20 °C. After 300 ml of synovial fluid had been processed the carboxymethyl-Trisacryl eluates were combined, dialysed against 50 mmol/l Tris-HCl, pH 7.0, 200 mmol/l NaCl, 5 mmol/l $CaCl_2$, 1 µmol/l $ZnCl_2$, 0.5 g/l $NaN_3$ (buffer B) and chromatographed on gelatin-Sepharose (4 cm × 3 cm) equilibrated in buffer B. Unbound protein was concentrated by ultrafiltration (Amicon, YM-10 membrane) and applied to Ultrogel AcA 44 (3 cm × 100 cm) equilibrated in buffer B. The activator-containing fractions were concentrated by ultrafiltration, dialysed against 20 mmol/l Tris-HCl, pH 8.0; 5 mmol/l $CaCl_2$, 1 µmol/l $ZnCl_2$, 0.5 g/l Brij 35, 0.5 g/l $NaN_3$ and applied to blue-Sepharose (2.5 cm × 7 cm) equilibrated in the same buffer. The activator was slightly retarded on blue-Sepharose, and activator-containing fractions were concentrated by ultrafiltration, dialysed against buffer A and applied to heparin-Ultrogel (1.5 cm × 8 cm) equilibrated with buffer A. The activator did not bind to heparin-Ultrogel and was further purified by gel filtration on a calibrated AcA 44 (2 cm × 100 cm) equilibrated in buffer B.

All following investigations of the matrix metalloproteinase activating properties of the activator were performed with polymorphonuclear leukocyte progelatinase.

### Demonstration of activator activity in SDS-PAGE

The procedures employed are essentially those of *Hibbs* et al. (9). To determine the polymorphonuclear leukocyte progelatinase activating activity of the protein seen in polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE), 1 µg activator was electrophoresed on a 12.5% gel. After electrophoresis, the gel was washed twice for 15 min in buffer B, pH 7.6, containing 25 g/l Triton X-100. After rinsing the gels briefly (5 min) in the above buffer without Triton X-100, the gel was sliced into 1 mm sections, and each slice was extracted with 200 µl buffer B for 24 h at 4 °C. 90 µl of each extract were mixed with 10 µl (2 µg) polymorphonuclear leukocyte progelatinase and 100 µl dinitrophenyl-labelled peptide and incubated at 37 °C. After 24 h the amount of hydrolysed substrate was determined.

To examine the gelatinase activity of the protein seen on SDS-PAGE, 60 ng activator were electrophoresed on a 10% SDS-PAGE, containing 2 g/l gelatin at 4 °C. The gel was washed as described above and the zymogram was developed in 50 mmol/l Tris-HCl, 5 mmol/l $CaCl_2$, 1 µmol/l $ZnCl_2$, 10 g/l Triton X-100, 0.2 g/l $NaN_3$, pH 7.6 for 24 h at 37 °C. The gels were stained with Coomassie brilliant blue.

### pH-Optimum

Five µl (1 µg) polymorphonuclear leukocyte progelatinase were incubated with 5 µl (15 ng) activator and 40 µl buffer B adjusted to pH 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0. After 1 h at 37 °C, 50 µl of a solution containing 200 mmol/l Tris-HCl, pH 7.0; 200 mmol/l NaCl, 5 mmol/l $CaCl_2$, 0.5 g/l $NaN_3$ and 100 µl dinitrophenyl-labelled peptide were added. After an incubation of 30 min at 37 °C the reaction was terminated and the amount of hydrolysed dinitrophenyl-labelled peptide was determined.

### Sensitivity to trypsin

Activator (6 µg) in 198 µl buffer B was incubated with 22 µl bovine trypsin (1 g/l) at 37 °C. Periodically 3.3 µl were taken and added to 5 µl aprotinin (1 g/l) and 81.7 µl buffer B to assay the activity against polymorphonuclear leukocyte progelatinase. To measure the activity against the dinitrophenyl-labelled pep-

tide, 25 µl of the mixture were added to 5 µl aprotinin and 70 µl buffer B. The reaction was started with either 100 µl dinitrophenyl-labelled peptide alone for 18 h (activity against dinitrophenyl-labelled peptide) or with the same substrate and additionally 10 µl (2 µg) polymorphonuclear leukocyte progelatinase for 30 min (activator-activity).

### Sensitivity to plasmin

The same procedure as described for the trypsin[1]) sensitivity was applied to the investigation on the activator sensitivity towards plasmin (1 g/l).

## Results

### Activator purification

The amount of activator that can be isolated from synovial rheumatoid fluid depends largely on the kind of treatment the patients received. Treatment with glucosteroids leads to a very low content or even a lack of metalloproteinases in the synovial fluid. Using only synovial fluid of patients without glucosteroid treatment we isolated usually 30–100 µg activator from 100 ml synovia.

Employing the method described, an activator for polymorphonuclear leukocyte progelatinase and polymorphonuclear leukocyte procollagenase with a molecular mass of $M_r$ 27 000 was purified from human rheumatoid synovia. No activator could be detected in synovial fluid itself. The activator is detectable usually after Ultrogel AcA 44 chromatography, sometimes earlier, after gelatin-Sepharose chromatography. Up to this purification step, identification of the activator is based on its ability to hydrolyse the dinitrophenyl-labelled peptide.

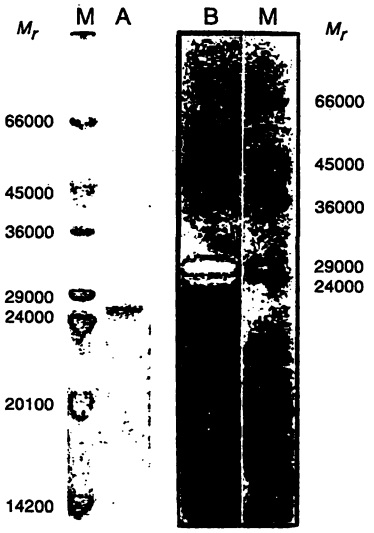


Fig. 1. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate and zymogram of purified activator.
Lane A: 1 µg activator on 12.5% polyacrylamide gel; electrophoresis in the presence of sodium dodecyl sulphate. Lane B: 60 ng activator on 10% polyacrylamide; gel electrophoresis in the presence of sodium dodecyl sulphate containing 2 g/l gelatin. Lane M: Molecular mass markers ($M_r$): bovine albumin (66 000), ovalbumin (45 000), glyceraldehyde-3-phosphate dehydrogenase (36 000), carbonic anhydrase (29 000), trypsinogen (24 000), trypsin inhibitor (20 100) and α-lactalbumin (14 200).

The relative molecular mass of the activator was determined in different ways: on a calibrated Ultrogel AcA 44 column the activator was eluted in the range $M_r$ 26 000–28 000, in 12.5% polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate a main band at $M_r$ 27 000 with one or two minor bands with lower relative molecular masses could be visualized and a gelatin-zymogram showed proteolytic activity at the same position (fig. 1). When larger quantities of activator were applied, an additional band at $M_r$ 25 000 was observed on the zymogram. Assay of activator in eluates of sliced 12.5% SDS-polyacrylamide gels showed activator activity associated with the region corresponding to $M_r$ 26 000–28 000.

### Activity towards polymorphonuclear leukocyte procollagenase and fibroblast $M_r$ 72 000 progelatinase

The activator quickly activated polymorphonuclear leukocyte procollagenase, as already described (13–15). Fibroblast $M_r$ 72 000 progelatinase was not activated.

### pH-Optimum

The activator shows a pH-optimum between pH 7.0 and 8.0 (data not shown).

### Inhibition of the activator

The activator was inhibited by EDTA and the activity could not be restored by dialysing against buffer B. It was also inhibited by a slight excess of the $M_r$ 72 000 progelatinase-TIMP-2 complex (fig. 2), which acts in the same manner as TIMP-2 (11).

### Trypsin-sensitivity

The activator displayed a pronounced sensitivity to trypsin; after 30 min incubation at 37 °C more than 80% of the activator-activity was lost. In contrast, under identical conditions, a 30 min incubation resulted in a threefold enhancement of the hydrolysis of the dinitrophenyl-labelled peptide (fig. 3). Treat-

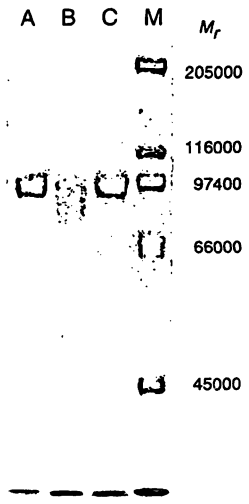


Fig. 2. 8% Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. Inhibition of activator by EDTA and by fibroblast $M_r$ 72 000 progelatinase-TIMP-2-complex.
2 µg polymorphonuclear leukocyte progelatinase in 10 µl buffer B were incubated for 45 min at 37 °C with 150 ng activator in 5 µl buffer B preincubated for 5 min at 37 °C with (Lane A) 800 ng $M_r$ 72 000 progelatinase-TIMP-2 complex in 20 µl buffer B, (Lane B) 20 µl buffer B, and (Lane C) 20 µl of 100 mmol/l Tris/HCl, 50 mmol/l EDTA, 200 mmol/l NaCl 0.5 g/l $NaN_3$, pH 7.0. (Lane M). Molecular mass markers ($M_r$): myosin (205 000), β-galactosidase (116 000), phosphorylase b (97 400), bovine albumin (66 000) and ovalbumin (45 000).

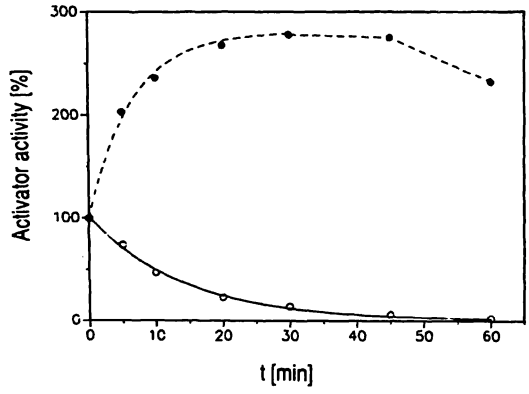


Fig. 3. Trypsin-sensitivity of the activator activity.
6 µg activator in 200 µl buffer B were incubated with 22 µg trypsin in 22 µl 1 mmol/l HCl at 37 °C. At the indicated times aliquots were withdrawn and activator activity towards polymorphonuclear leukocyte progelatinase (open circles) and dinitrophenyl-labelled peptide (solid circles) was determined.

ment with trypsin for 25 min at 37 °C did not alter the proteolytic activity of the activator against casein and gelatin (fig. 4). The action of trypsin reduced the molecular mass of the activator by $M_r$ 1000–2000, as shown by Western-blot analysis (fig. 5). Prolonged incubation with trypsin led to the destruction of the activator.

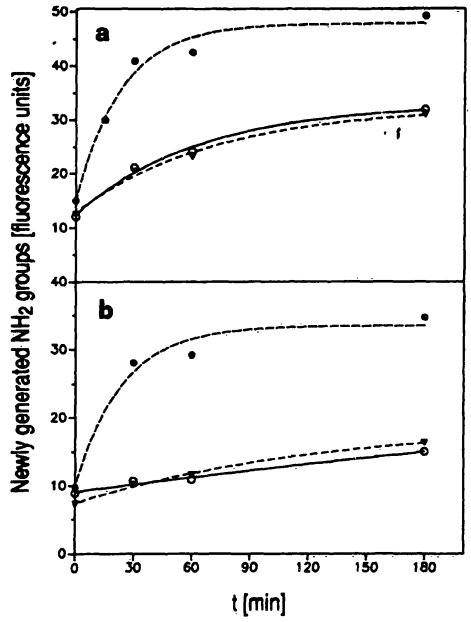


Fig. 4. Hydrolysis of casein (a) and gelatin (b) by the activator, trypsin-treated activator and by trypsin.
100 µg gelatin or casein, respectively, were incubated in a total volume of 200 µl buffer B with 1.2 µg activator (open circles), 1.2 µg activator preincubated with trypsin at 37 °C (solid triangles) or with 1.3 µg trypsin (solid circles). At the indicated times newly generated amino groups were determined with the fluorescamine method. The tryptic treatment of the activator was stopped with 6 µg aprotinin after 25 min.

## Plasmin-sensitivity

Under the conditions employed plasmin had no effect on the activator (data not shown). Even an incubation of 15 h at 37 °C did not change the activator's activity towards polymorphonuclear leukocyte gelatinase or the dinitrophenyl-labelled peptide.

## Activation of polymorphonuclear leukocyte gelatinase

The treatment of polymorphonuclear leukocyte progelatinase with the activator led via an $M_r$ 86 000 intermediate to an active gelatinase with a molecular mass of $M_r$ 82 000, (fig. 6) exactly as described for the action of stromelysin on polymorphonuclear leukocyte progelatinase (6).

## Substrate specificity

The specific activity of the activator against the dinitrophenyl-labelled peptide was 40 mU/mg, which is about 4% of polymorphonuclear leukocyte gelatinase activity against that substrate (11). It can be deduced from figure 4 that the activator's activity against casein is about 3 times higher than against gelatin. Compared with trypsin, however, the activator's activity towards these substrates is rather low, i.e. less than 10% of the trypsin activity towards gelatin and

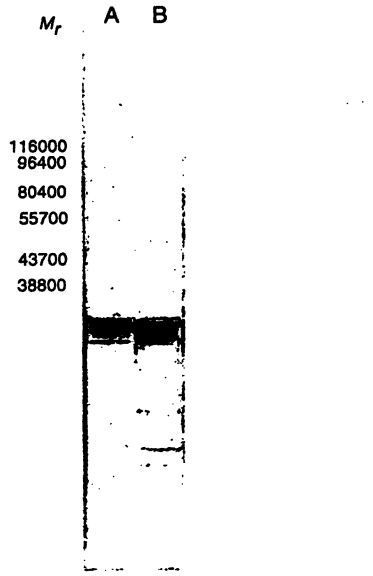


Fig. 5. Immunoblotting of activator and of trypsin treated activator.
600 ng activator in 20 µl buffer B (Lane A) and 600 ng activator in 20 µl buffer B incubated with 2 µg trypsin in 2 µl 1 mmol/l HCl at 37 °C for 30 min (Lane B) were run on a 12.5% polyacrylamide gel in the presence of sodium dodecyl sulphate, blotted on a Millipore Immobilon membrane and blocked overnight with bovine serum albumin. The blots were incubated with an antiserum raised in sheep against human stromelysin, followed by peroxidase-labelled anti-sheep-IgG. The reactive bands were visualized with 4-chloro-1-naphthol. The tryptic treatment was stopped with 2 µg aprotinin in 2 µl distilled water. The position of the apparent molecular mass for prestained protein is indicated: β-galactosidase (116000), fructose-6-phosphate kinase (96400), pyruvate kinase (80400), ovalbumin (55700), lactic dehydrogenase (43700) and triosephosphate isomerase (38800).

about 20% of that towards casein. Trypsin and activator are compared with respect to their polymorphonuclear leukocyte progelatinase activating properties in table 1, where it can be seen that the relationship is reversed, i.e. the activator is nearly 30 times more efficient than trypsin in the activation of polymorphonuclear leukocyte progelatinase.

Tab. 1. Activation of polymorphonuclear leukocyte progelatinase by activator and trypsin
2 µg polymorphonuclear leukocyte progelatinase in 100 µl buffer B and varying amounts of activator displaying a molecular mass of $M_r$ 27000 or bovine trypsin displaying molecular mass of $M_r$ 24000, respectively, were mixed with 50 µg dinitrophenyl-labelled peptide in 100 µl buffer B and incubated for 30 min at 37 °C.

| Activator (ng/200 µl) | Substrate hydrolysed (nmol) | Trypsin (ng/200 µl) | Substrate hydrolysed (nmol) |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 30 | 3.8 | 600 | 2.5 |
| 45 | 5.5 | 1200 | 5.2 |
| 60 | 7.8 | 1800 | 7.9 |
| 75 | 9.9 | 2400 | 11.2 |
| 90 | 12 | — | — |

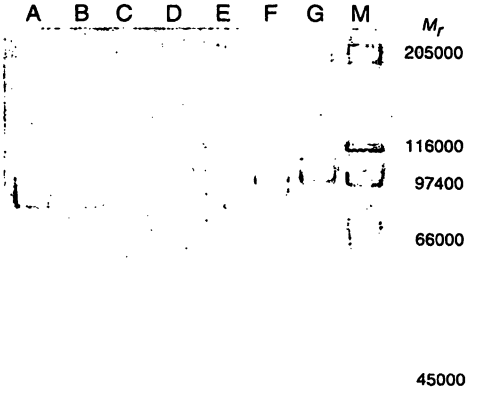


Fig. 6. Activation of polymorphonuclear leukocyte progelatinase with activator.
1 µg polymorphonuclear leukocyte progelatinase in 5 µl buffer B was incubated with 30 ng activator in 1 µl buffer B for 60 (Lane A), 45 (Lane B), 30 (Lane C), 20 (Lane D), 10 (Lane E), 5 (Lane F) and 0 (Lane G) min, respectively, and analysed on 8% polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. Lane M: molecular mass markers ($M_r$): myosin (205000), β-galactosidase (116000), phosphorylase 97400), bovine albumin (66000) and ovalbumin (45000).

## Discussion

From the synovial fluid of patients suffering from rheumatoid arthritis, we isolated an activator for polymorphonuclear leukocyte procollagenase and polymorphonuclear leukocyte progelatinase, with a relative molecular mass of $M_r$ 27000. Furthermore, we demonstrated that the activator is an active form of the matrix metalloproteinase stromelysin; the activator is inhibited by EDTA and the $M_r$ 72000 gelatinase-TIMP-2 complex, which acts as an inhibitor for matrix metalloproteinases the same way as TIMP (11), thereby establishing that the activator is a member of the family of matrix metalloproteinases. The activator reacts with antibodies raised against human stromelysin, its pH-optimum lies in the neutral range, and it splits the polymorphonuclear leukocyte gelatinase in a manner characteristic of stromelysin.

The activator was not only found in synovia of patients suffering from rheumatoid arthritis but also in the synovia of patients with osteoarthritis or systemic lupus erythematosus during an inflammatory attack. The activator could not be detected in synovia from non-inflammatory joints, e.g. distorsion of the knee.

Synovia subsequently found to contain a high concentration of activator also displayed a general enhancement of matrix metalloproteinase activity. Activator activity was not detectable in synovial fluids but became apparent after the chromatographic step

on Ultrogel AcA 44, sometimes earlier, after gelatin-Sepharose. Latent prostromelyin is therefore activated during the purification procedure. Whether this is due to the action of other proteinases or to an autocatalytic process is still unclear.

We believe that an activator for polymorphonuclear leukocyte collagenase and polymorphonuclear leukocyte gelatinase that was isolated from synovial fluid (12–14) and from the culture media of synoviocytes (15) many years ago is also stromelysin. However, this activator had a reported relative molecular mass of $M_r$ 35 000 and it lacked proteolytic activity against casein and gelatin. We could not detect activator activity in the relative molecular mass range of $M_r$ 35 000, either on a calibrated Ultrogel AcA 44, or by eluting protein from sliced SDS-polyacrylamide electrophoresis gels.

Polymorphonuclear leukocyte progelatinase, polymorphonuclear leukocyte procollagenase, fibroblast $M_r$ 72 000 progelatinase, casein, gelatin and dinitrophenyl-labelled peptide were examined as substrates for the proteolytic activity of the activator. With the exception of the fibroblast $M_r$ 72 000 progelatinase, the activator hydrolysed all these substrates, but showed a rather high degree of specificity for polymorphonuclear leukocyte progelatinase and polymorphonuclear leukocyte procollagenase. The activator was much less active than trypsin in the proteolysis of casein and gelatin. However, although trypsin is a potent activator for polymorphonuclear leukocyte progelatinase (16), the stromelysin activator is about 30 times more efficient. The activator displays a similar efficiency in the activation of polymorphonuclear leukocyte collagenase, while the fibroblast $M_r$ 72 000 progelatinase is not a substrate for the activator. The high specificity for the matrix metalloproteinases displayed by neutrophilic granulocytes may indicate a physiological role of this activator in the activation of these proteinases. This specificity may also explain why the $M_r$ 35 000 activator described earlier was not found to hydrolyse gelatin and casein; activator concentrations too low to significantly hydrolyse gelatin and casein are still sufficient to rapidly activate the polymorphonuclear leukocyte progelatinase.

Hydrolysis of the dinitrophenyl-labelled peptide by the activator was slow compared with the rate of hydrolysis by polymorphonuclear leukocyte gelatinase. However, a short tryptic treatment, that did not lead to a significant change in the molecular mass of the activator (fig. 5), enhanced the activator's activity against the dinitrophenyl-labelled peptide about threefold, destroyed its ability to activate polymorphonuclear leukocyte progelatinase and did not affect its activity against casein and gelatin. It might therefore be concluded that a similar physiological proteolytic event may significantly increase the activator's activity against some components of the extracellular matrix. Plasmin, a proteinase with proteolytic properties similar to trypsin, plays an important role under physiological conditions in the activation of procollagenase (MMP-1) and prostromelysin, but it had no effect on the activator.

It could be argued that the three different trypsin sensitivities of the activator might be due to the presence of two different proteinases that react with trypsin in different ways:

1. the activator (active stromelysin) which hydrolyses the dinitrophenyl labelled peptide, casein/gelatin and the polymorphonuclear leukocyte progelatinase, and which is inactivated by trypsin;
2. a latent metalloproteinase which, after activation by trypsin, could be responsible for the enhanced turnover of the dinitrophenyl labelled peptide, and which at the same time compensates the loss of the caseinolytic/gelatinolytic activity of the activator. According to the relative molecular mass this metalloproteinase could only be matrilysin (MMP-7), but so far this enzyme has only been found in the uterus (17) and in developing macrophages (18). In addition, in synovial fluid, no such small latent metalloproteinase could be detected after chromatography on Ultrogel AcA 44 (not shown).

The distinct sensitivity to trypsin of the activator's proteolytic activity towards different substrates suggests that during the transformation from prostromelysin to active stromelysin at least one switch position is passed. At this switch position a putative regulatory mechanism may have the option of quickly deciding whether stromelysin should function as an activator for matrix metalloproteinases, or concomitantly, with enhanced activity, as a proteinase of other substrates.

Recently, it was reported that treatment of prostromelysin with *p*-aminophenylmercury acetate led to the formation of an activator for polymorphonuclear leukocyte progelatinase with a relative molecular mass of $M_r$ 47 000 (6). This activator was not investigated with regard to its sensitivity to trypsin and it would be interesting to know if this $M_r$ 47 000 activator behaves in the same way as the $M_r$ 27 000 activator described in the present report.

The activity of matrix metalloproteinases in the extracellular space is subject to regulation on at least three different levels — expression, activation, and interaction with specific inhibitors. The present data

extend this regulatory repertoire by the observation that stromelysin may be further processed to active species displaying distinct and separate functions as activators or hydrolytic enzymes of macromolecules of the extracellular matrix.

We are now investigating whether a mechanism is present in the synovial fluid of patients afflicted by rheumatoid arthritis, which under physiological conditions may initiate a cascade of events, producing and processing stromelysin as described here in vitro.

**Acknowledgement**

This work was supported by Hoechst AG, Frankfurt am Main, Germany.

## References

1. Matrisian, L. M. (1992) The matrix-degrading metalloproteinases. BioEssays *14*, 455–463.
2. Birkedal-Hansen, H., Werb, Z., Welgus, H. & Van Wart, H. (eds.) (1992) *Matrix Metalloproteinases and Inhibitors* Gustav Fischer, Stuttgart, Jena, New York.
3. Murphy, G., Cockett, M. I., Stephens, P. E., Smith, B. J. & Docherty, A. J. P. (1987) Stromelysin is an activator of procollagenase. A study with natural and recombinant enzymes. Biochem. J. *248*, 265–268.
4. Ito, A. & Nagase, H. (1988) Evidence that human rheumatoid synovial matrix metalloproteinase 3 is an endogenous activator of procollagenase. Arch. Biochem. Biophys. *267*, 211–216.
5. He, Ch., Wilhelm, S. M., Pentland, A. P., Marmer, B. L., Grant, G. A., Eisen, A. Z. & Goldberg, G. J. (1989) Tissue cooperation in a proteoolytic cascade activating human interstitial collagenase. Proc. Natl. Acad. Sci. USA *86*, 2632–2636.
6. Ogata, Y., Enghild, J. J. & Nagase, H. (1992) Matrix metalloproteinase 3 (stromelysin) activates the precursor of the human matrix metalloproteinase 9. J. Biol. Chem. *267*, 3581–3584.
7. Masui, Y., Takemoto, T., Sakakibara, S. & Hori, H. (1977) Synthetic substrates for vertebrate collagenase. Biochem. Med. *17*, 215–221.
8. Macartney, H. W. & Tschesche, H. (1980) Latent collagenase from human polymorphonuclear leukocytes and activation to collagenase by removal of an inhibitor. FEBS Lett. *119*, 327–332.
9. Hibbs, M. S., Hasty, K. A., Seyer, J. M., Kang, A. H. & Mainardi, C. L. (1985) Biochemical and immunological characterization of the secreted forms of human neutrophil gelatinase. J. Biol. Chem. *260*, 2493–2500.
10. Hasty, K. A., Hibbs, M. S., Kang, A. H. & Mainardi, C. L. (1986) Secreted forms of human neutrophil collagenase. J. Biol. Chem. *261*, 5645–5650.
11. Kolkenbrock, H., Orgel, D., Hecker-Kia, A., Noack, W. & Ulbrich, N. (1991) The complex between a tissue inhibitor of metalloproteinases (TIMP-2) and 72-kDa progelatinase is a metalloproteinase inhibitor. Eur. J. Biochem. *198*, 775–781.
12. Kruze, D. & Wojtecka, E. (1972) Activation of leucocyte collagenase proenzyme by rheumatoid synovial fluid. Biochim. Biophys. Acta *285*, 436–446.
13. Wize, J., Sopata, I., Wojtecka-Lukasik, E., Ksiezny, S. & Dancewicz, A. M. (1975) Isolation, purification and properties of a factor from rheumatoid synovial fluid activating the latent forms of collagenolytic enzymes. Acta Biochimica Polonica *22*, 239–250.
14. Dancewicz, A. M., Wize, J., Sopata, I., Wojtecka-Lukasik, E. & Ksiezny, S. (1978) Specific and nonspecific activation of latent collagenolytic proteases of human polymorphonuclear leukocytes. In: *Neutral Proteases of Human Polymorphonuclear Leukocytes* (Havemann, K. & Janoff, A., eds.) pp. 373–383, Urban & Schwarzenberg, Inc. Baltimore–Munich.
15. Wize, J., Abgarowicz, T., Wojtecka-Lukasik, E., Ksiezny, S. & Dancewicz, A. M. (1975) Activation of human leucocyte procollagenase by rheumatoid synovial tissue culture medium. Ann. Rheum. Dis. *34*, 520–523.
16. Kolkenbrock, H., M.-Ali, H., Hecker-Kia, A., Buchlow, G., Sörensen, H., Hauer, R. W. & Ulbrich, N. (1991) Characterization of a Gelatinase from Human Rheumatoid Synovial Fluid Cells. Eur. J. Clin. Chem. Clin. Biochem. *29*, 499–505.
17. Woessner, J. F. jr. & Taplin, C. J. (1988) Purification and properties of a small latent matrix metalloproteinase of the rat uterus. J. Biol. Chem. *263*, 16918–16925.
18. Busiek, D. F., Ross, F. P., McDonnell, S., Murphy, G., Matrisian, L. M. & Welgus, H. G. (1992) The matrix metalloproteinase matrilysin (PUMP) is expressed in developing human mononuclear phagocytes. J. Biol. Chem. *267*, 9087–9092.

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